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Observation of microbial mats from drainpipe at the landslide area in Takayanagi, Niigata, Japan - Diversity of ecosystem depends on the characteristics of water -

Hiromi Segawa[1], Kazuhiro Sato[1], Shuichi Shiraishi[2], Naoto Takahashi[2], Takaaki Kanemoto[2] and Kazue Tazaki[3]

[1]*Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192 Japan*

[2]*Nissaku co. Ltd., Niigata Branch, Jokido, Niigata 950-0891 Japan*

[3]*Department of Earth Sciences, Faculty of Science, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192 Japan*

Abstract

The diversity of environmental ecosystem was found in the landslide area of Takayanagi, Niigata, Japan. Where a well-drained drainage system was established in March 1991. Each drainpipe produced groundwater to form microbial mats. Optical microscopic observation of different colored biomats revealed the microbial diversity in short interval drainpipes. Mainly filamentous bacteria with diatom were observed in the 1st point of drainage, while the 2nd point showed the presence of *Leptothrix ochracea* with algal filaments. In the 3rd point, diatoms were found covered with numerous tubular bacteria associated with algal filaments. Furthermore, green microbial mats in the 4th point contain various diatom and algal filaments. Thus, the results of microbial diversity in different water conditions, such as pH, Eh, EC, DO and water temperature, suggesting that characteristics of water have a great influence in the diversity of ecosystem. Increase of Eh, DO and WT encourage propagating of diatoms and algae in the drainpipes.

Key words: Diversity of environmental ecosystem, Landslide area, Characteristics of water, Microbial mats, Diatom, *Leptothrix ochracea*, Algae

INTRODUCTION

Microorganisms in microbial mats can grow and survive in the natural environment including some of the most extreme and adverse environment on the Earth, such as high pollution, high temperature and high pressure even in the strong acidic and heavy metal rich condition (Fyfe 1997; Tazaki 1999). Recently, in the most of cases microorganisms have been used as indicators for the investigation of environment (Khristoforova 1999). The precipitation of minerals by living organisms is widespread phenomenon that is generally referred to as biomineralization (Ferris 1993). The significance of bacterial biomineralization is emphasized by the fact that microorganisms are the most abundant life form on the surface of the earth and live in a variety of environments (Ferris and Beveridge 1985). In natural environments, such as pond, stream, hot springs, and drainage system, biomats are often formed. Diatoms and bacteria usually involved in these formation processes. Diatoms precipitate silicon on their cell walls, whereas bacteria can accumulate Fe, Mn, Sr, and some other elements in/on to the cells (Beveridge 1989). Chemical and biological diversity of biomats influences water ecosystem (Tazaki 2000). Takayanagi in Niigata prefecture is a typical landslide area for microbial study, where the drainage systems were established to prevent mudslide occurrence. There, biomats were found predominating in the drainpipe outlet. For this reason, it might be considered that biomats those consisted of microorganism are able to indicate the conditions of the environment at the locality.

In this study, microscopic observation of different colored biomats collected from the drainpipes with a short interval at the landslide area revealed the diversity of ecosystem, which depends on the characteristics of water.

MATERIALS AND METHODS

Water drainage system (through the pipe) was established in the landslide areas of Takayanagi, Niigata, Japan in March of 1991 (Fig. 1). The numerous drainpipes were set up in the locality to inhibit mudslide occurrence. Each drainpipe carried groundwater resulting the formation of microbial mats in the outlet of the pipes.

Different colored biomats with ground waters were collected from those drainpipe outlets for analysis on the 13th September 2002 (Fig. 2, point 1, 2, 3, and 4). Six

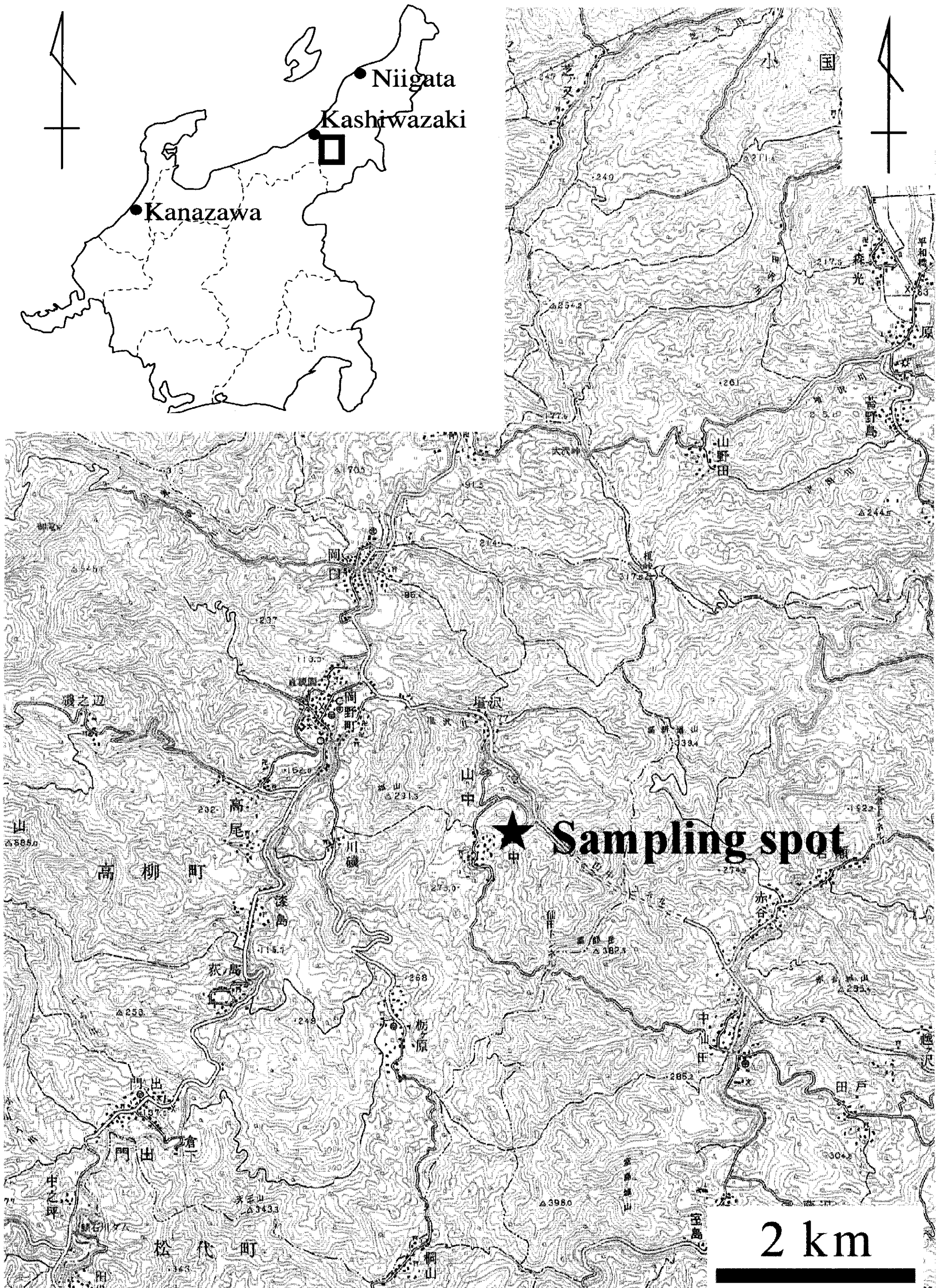

Fig. 1 Locality map of sampling spot at the landslide area in Takayanagi, Niigata, Japan.

drainpipes, 50 m in length, were radially set up at the sampling points for drained groundwater off. Each of the drainpipes have a short interval among them about 50 cm. The biomats are light reddish brown (point 1), reddish brown (point 2), greenish brown (point 3), and green biomats (point 4) in color, respectively.

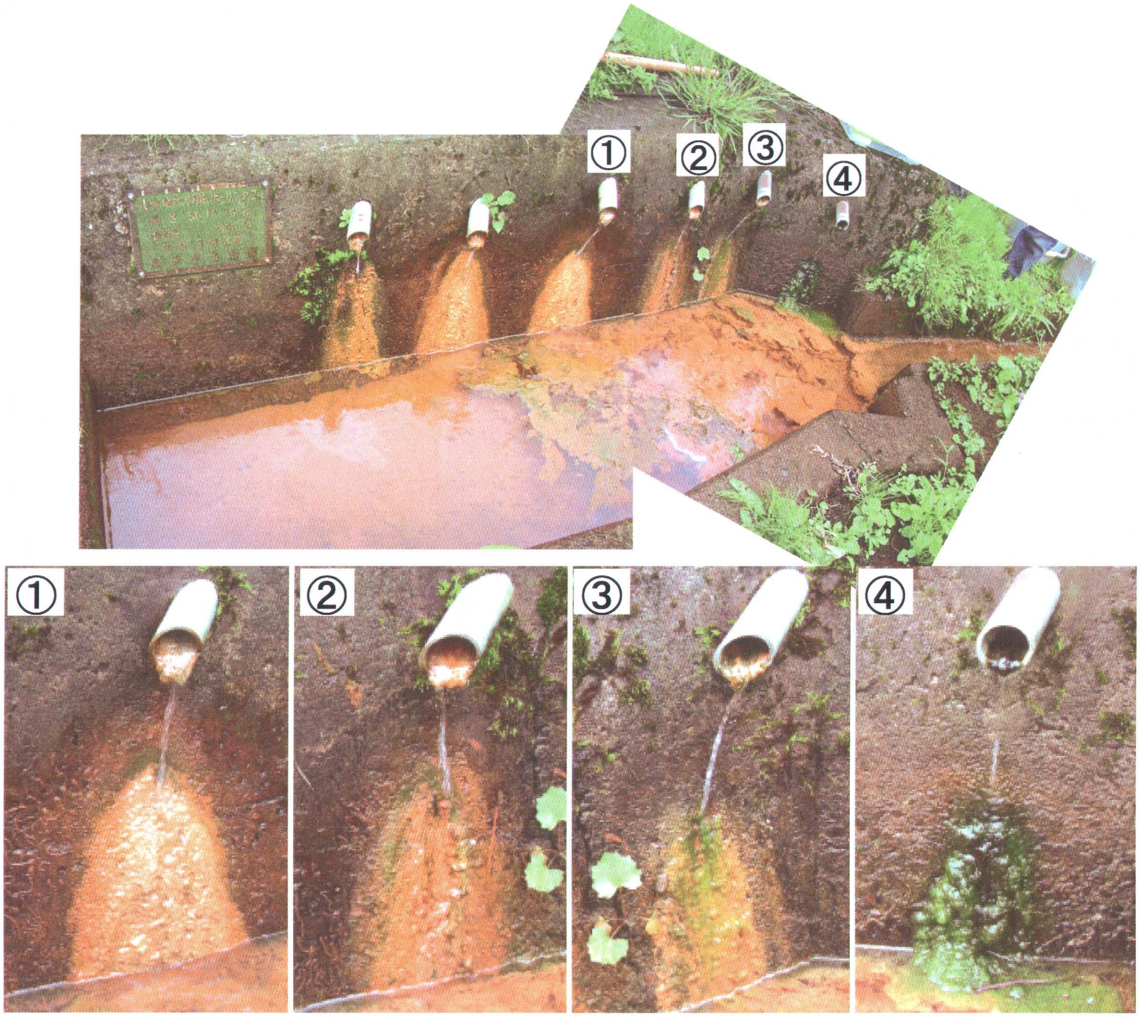

Fig. 2 Field views of biomats in drainpipe outlet at the landslide area in Takayanagi showing light reddish brown biomats (①), reddish brown biomats (②), greenish brown biomats (③), and green biomats (④).

The pH, Eh, EC, DO and WT were measured in the field by a portable water quality inspection meter (pH; D-21 made by HORIBA, Eh; D-13 made by HORIBA, EC; ES-12 made by HORIBA, DO and WT; DO21P made by TOA). Furthermore, to identify the presence and diversity of microorganism optical microscopic observation was carried out. Hand picked biomats were mounted on and spread over the slide simultaneously. Biomats were stained with 4’6-diamidino-2-phenylindole (DAPI, 50 μg/ml) to observe under epifluorescent microscope (Nikon EFD 3, Digital camera: COOLPIX 995). For the epifluorescent microscopic observation, a filter UV-1 A (wavelength of exposed light: 360-370 nm) was used.

RESULTS AND DISCUSSION

The ground water quality and the types of microorganisms are given in Table 1. The pH was found relatively constant (6.2 ~ 6.5) in groundwater, whereas Eh, DO, and WT had been increased gradually from point 1 to 4. On the other hand only EC was found decreased in point 2, 3, and 4 compared with that of point 1. Eh and DO results showed the relatively increased oxidative condition in waters between points 1 ~ 4. Especially, point 4 showed the highest oxidative condition of water. Additionally it might be suggested that the reason of green biomats formation depends on the high oxidative condition of water.

Table 1 Characteristics of water and biomats collected from drainpipe at the landslide area in Takayanagi, Niigata, Japan.

Sampleing point	pH	Eh(mV)	EC(mS/m)	DO(mg/l)	WT (℃)	Types of microorganism
1	6.5	141	29.8	2.1	13.0	filamentous bacteria, diatom, coccus and bacilus bacteria
2	6.4	190	22.3	3.4	14.9	**Leptothrix ochracea*, algae, diatom, coccus and bacilus bacteria
3	6.2	303	19.4	3.8	15.3	tubular bacteria, algae, diatom, coccus and bacilus bacteria, *Leptothrix ochracea*
4	6.3	456	25.8	6.7	17.2	variety of diatoms, algae, coccus and bacilus bacteria

13th September 2002, mesurement
* Similarity with the Holt et al. (1994)

The optical microscopic observation of biomats collected from four drainpipe outlets indicated the diversity of ecosystem depends on the characteristics of water. The details are as follows according to the points of sampling.

General view of light reddish brown biomats collected from sampling point 1, seen under the optical microscope, indicated that they were consisted mainly of filamentous bacteria (Fig. 3 ①-a and ①-b). Filamentous bacteria associated with diatom, coccus and bacillus types of bacteria, which formed a network structure. Both of the filamentous bacteria and diatoms were of about 20 μm in size, where diatoms contained chlorophyll inside of their cells (Fig. 3 ①-c and ①-d).

General view of the reddish brown biomats collected from point 2 in Fig. 2, showed the specific tubular bacteria forms a network structure with the reddish brown

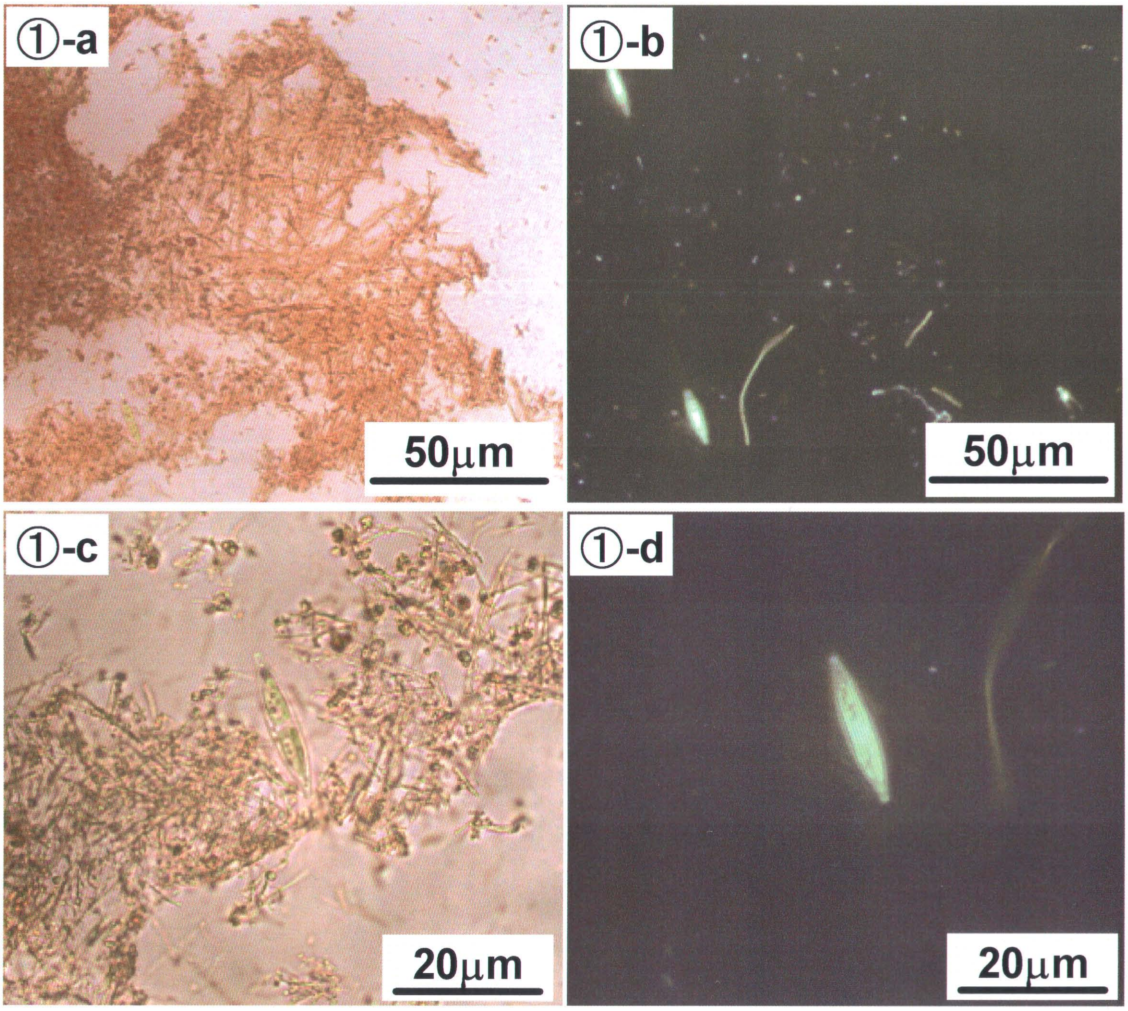

Fig. 3 Optical micrographs of the light reddish brown biomats collected from the drainpipe outlet (in point 1 of Fig. 2) of a landslide area in Takayanagi. Filamentous bacteria formed network structure associated with a few diatoms which contained chlorophyll, coccus and bacillus types of bacteria (①-a; light, ①-b; DAPI staining epifluorescent view). Magnified views of ①-a and ①-b indicate the size of diatoms and filamentous bacteria; both of them are of about 20 μm in size (①-c; light, ①-d; DAPI staining epifluorescent view).

materials (Fig. 4 ②-a). The shape (tubular) and size (50-200 μm in length, 2 μm in wide) of the microorganism confirmed the presence of the iron bacteria, *Leptothrix ochracea* associated with algal filaments, diatoms, and bacteria of filamentous, coccus and bacillus types (Fig. 4 ②-b). Magnified view of Fig. 4 ②-a showed *Leptothrix ochracea* adhered with yellowish brown particles, which might be considered as iron oxides (Fig. 4 ②-c).

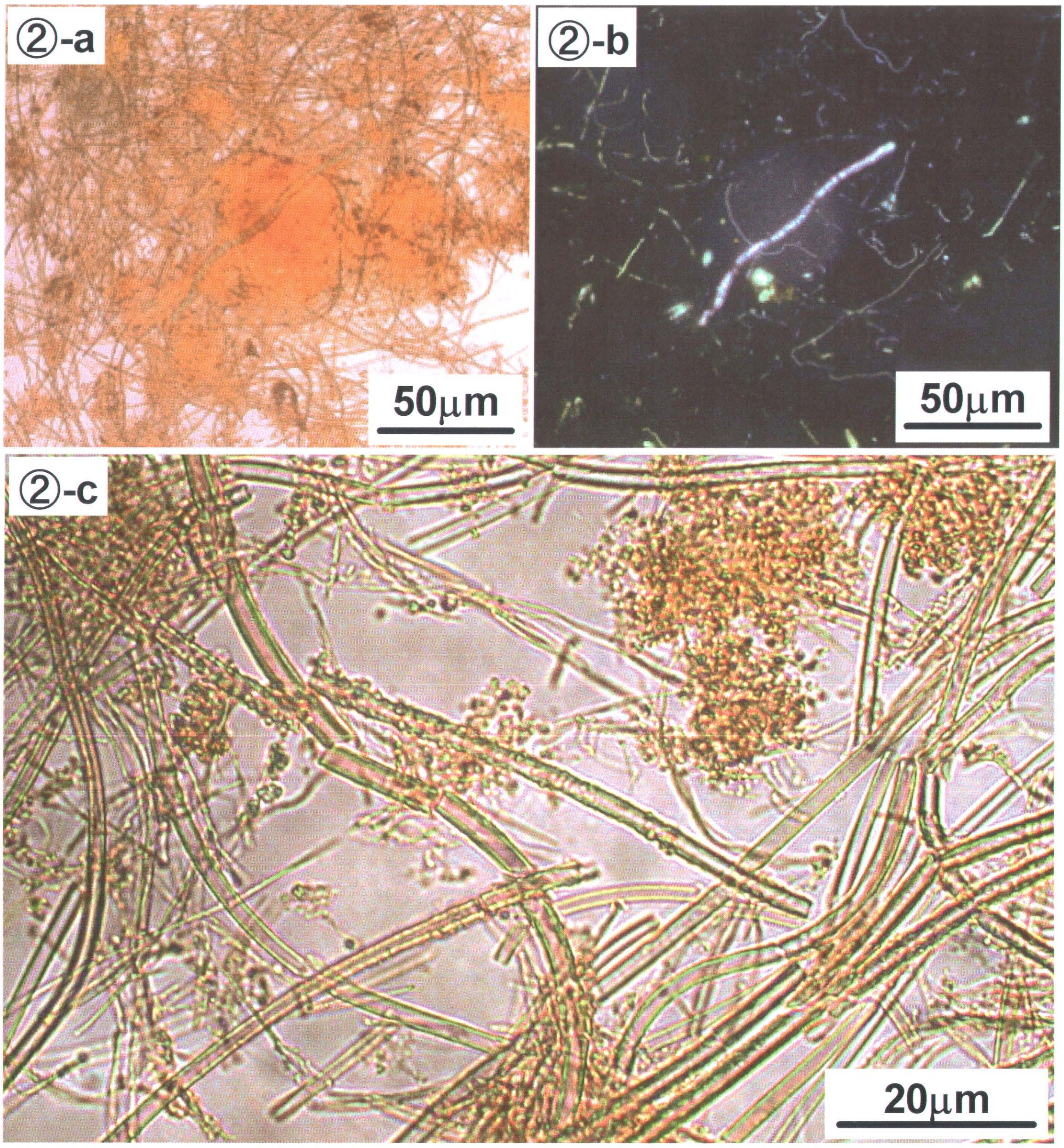

Fig. 4 Optical micrographs of the reddish brown biomats collected from the drainpipe outlet (in point 2 of Fig. 2) of a landslide area in Takayanagi. Iron bacteria, *Leptothrix ochracea,* formed network structure with reddish brown materials (②-a; light, ②-b; DAPI staining epifluorescent view). Magnified view of ②-a shows the network structure of *Leptothrix ochracea* adhered with yellowish brown particles (②-c).

Furthermore, greenish brown biomats (point 3 of Fig. 2) was consisted mainly of tubular bacteria and diatoms of about 20 μm in size (Fig. 5 ③-a, ③-b, ③-c, and ③-d). Tubular bacteria have the similar shape with different sizes to that of *Leptothrix ochracea*. However, the size of these bacteria was a little smaller (20 μm) than

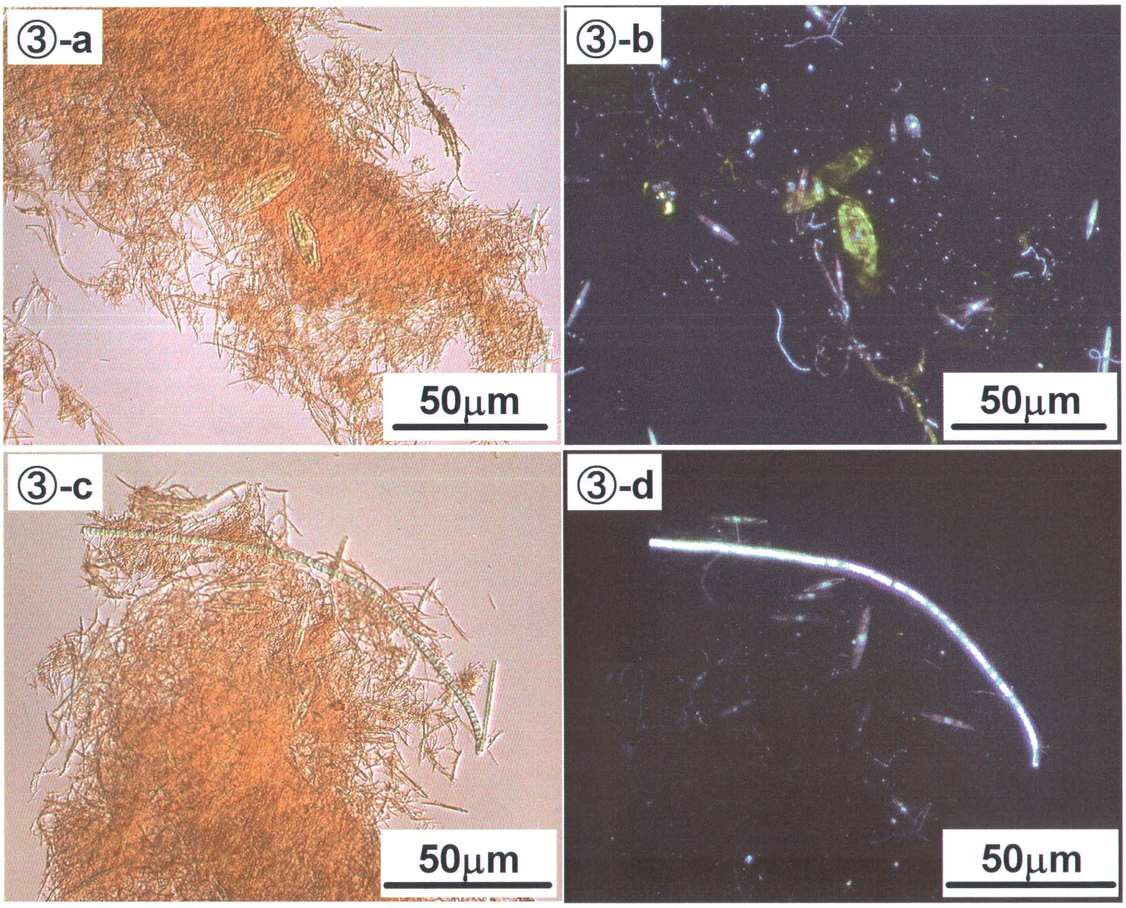

Fig. 5 Optical micrographs of the greenish brown biomats collected from the drainpipe outlet (in point 3 of Fig. 2) of a landslide area in Takayanagi. Tubular bacteria have been found associated with algal filaments and diatoms (③-a; light, ③-b; DAPI staining epifluorescent view). The size of tubular bacteria and diatoms; both of them are of about 20 μm in size (③-c; light, ③-d; DAPI staining epifluorescent view).

Leptothrix ochrecea. The numerous tubular bacteria covered the photosynthetic algal filaments and diatoms (Fig. 5 ③-a, ③-b, ③-c, and ③-d). Naturally, photosynthetic diatom and algae used to produce oxygen. The reason of tubular bacterial covering on to the photosynthetic algae or diatom might be the oxidative condition of their cell surface (algae and diatom), which are capable to accelerate iron oxidation. The result consistent with parts of Kenneth (1983) work.

General view of the green biomats collected from point 4 in the sampling locality showed the presence of various kinds of diatoms, bacteria and algae (Fig. 6 ④-a and ④-b). A group of diatom (Fig. 6 ④-c and ④-d) covered with a thin film like mucus layer, which might have the similarity with the meiotic cell division of *Craticula*

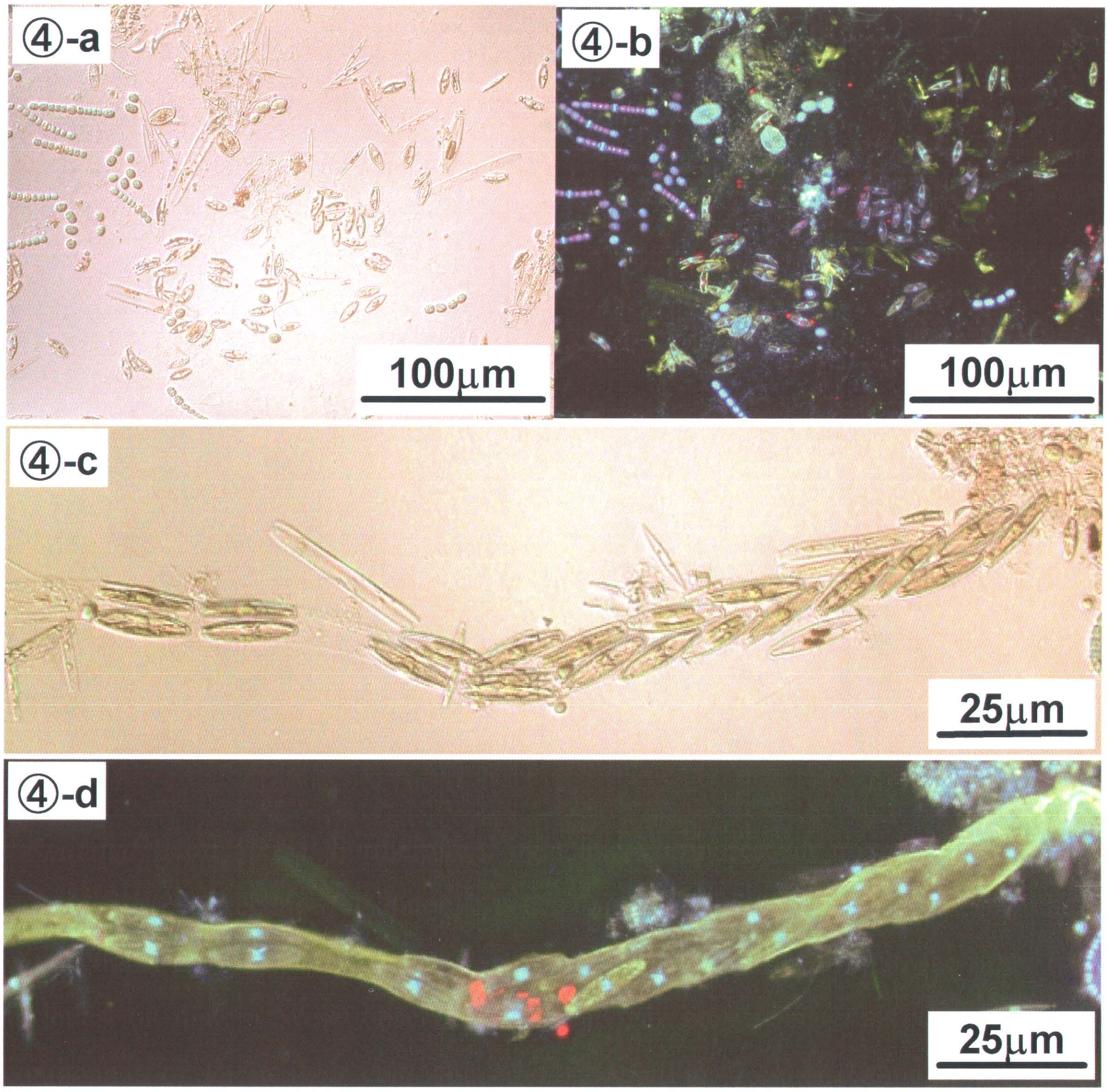

Fig. 6 Optical micrographs of the green biomats collected from the drainpipe outlet (in point 4 of Fig. 2) of a landslide area in Takayanagi. General view of biomats sample shows the presence of various kind of diatom associated with bacteria and algae (④-a; light, ④-b; DAPI staining epifluorescent view). A group of diatom covered with thin films (④-c; light, ④-d; DAPI staining epifluorescent view).

cuspidatea (Hori 1993). Furthermore, light and epifluorescent micrographs of same sample show aggregation of diatoms, coccus and bacillus types of bacteria associated with reddish brown materials (Fig. 7 ④-e, ④-f, ④-g, and ④-h). On the other hand varieties of diatom were observed with reddish brown materials that might be compounds of Fe in their cells (Fig. 7 ④-i and Fig. 7 ④-j). Kuma *et al.* (1999) suggested that diatom were able to uptake organic-Fe (Ⅲ) from the environment.

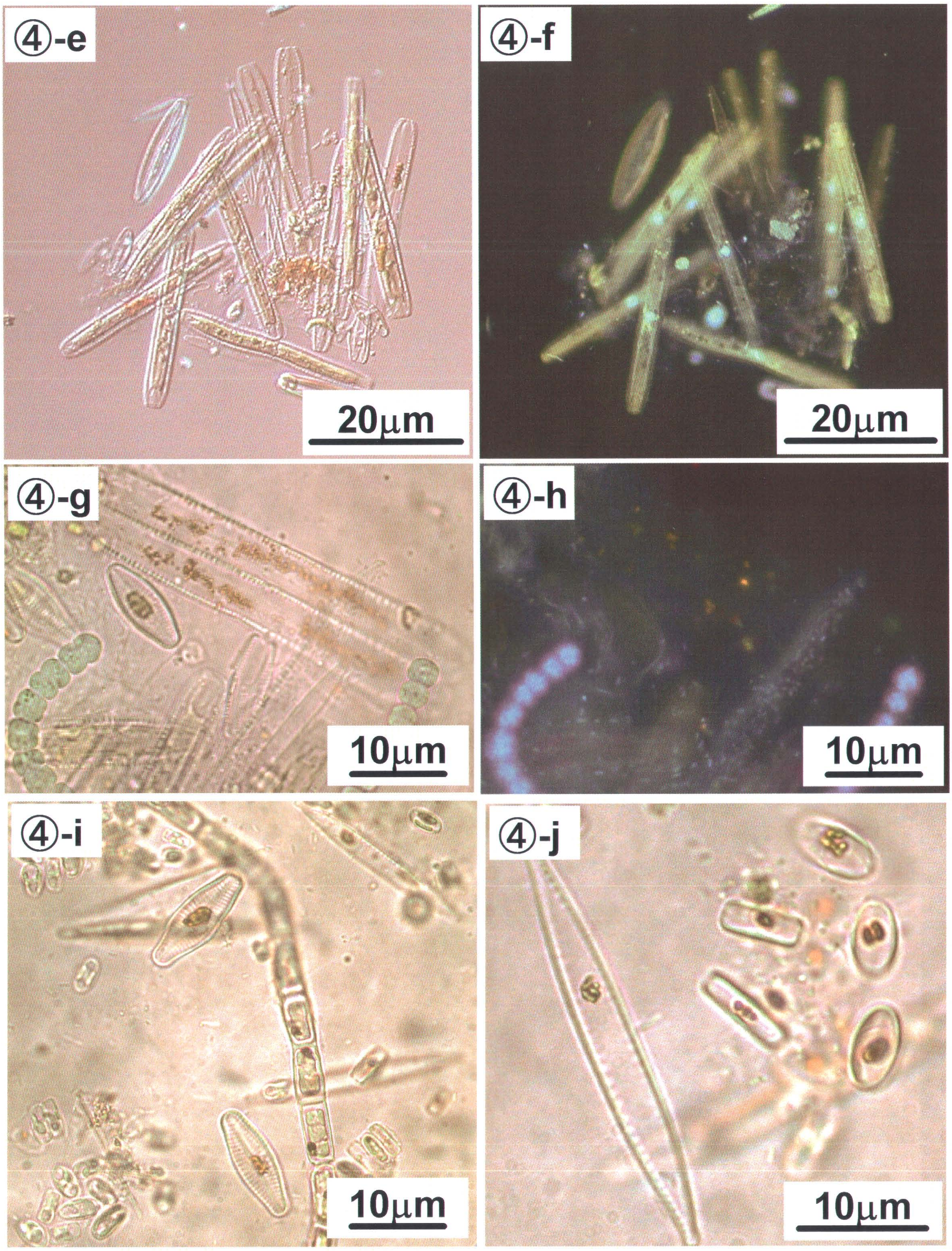

Fig. 7 Optical micrographs of the green biomats collected from the drainpipe outlet (in point 4 of Fig. 2) of a landslide area in Takayanagi. Micrographs show the presence of various kinds of diatoms, coccus and bacillus types of bacteria (④-e and ④-g; light, ④-f and ④-h; DAPI staining epifluorescent view). Reddish brown materials were found inside of diatom cells (④-i and ④-j).

Koiwasaki (1996) reported from a mineralogical study of diatom that can take part in the formation of biominerals associated with Fe, As, and S.

Microorganisms in biomats have their own niche in the geo-aquatic ecosystem. They could accumulate various kinds of elements like Fe, Si, etc. from the environment. In this study, *Leptothrix ochrecea,* a kind of iron bacteria, was found active in the low temperature under reductive condition. Basing on the results of water quality and microscopic observations, it seem that the water characteristics and microbial diversity in different colored biomats indicate the diversity of environmental ecosystem.

CONCLUSION

The diversity of environmental ecosystems in drainpipe outlet was found in the landslide area in Takayanagi, Niigata Japan. Optical microscopic observation of different colored biomats revealed the microbial diversity in a short interval. The results suggest that characteristics of water have a great influence in the diversity of environmental ecosystem.

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